Further evidence for Introgression of Pinus taeda with P. echinata: electrophoretic variability and variation in resistance to Cronartium fusiforme[1])

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Summary

Twenty-nine single tree selections of loblolly (*Pinus taeda,)* shortleaf *(P. echinata),* and suspected hybrid pines were made from natural populations in the species' western-most distributions (East Texas). Electrophoretic assays of general proteins yielded 13 unique, electrophoretic phenotypes which were compared to the response exhibited by the open-pollinated progeny families to inoculation by *Cronartium fusiforme* (fusiform rust). Results suggest that introgression of the two pine species has occurred and that maternal morphology and electrophoretic phenotype represent reasonable methods for predicting progeny response to fusiform rust among sympatric loblolly-shortleaf pine populations in East Texas.

Key words: introgressive hybridization, fusiform rust, electrophoresis, *Pinus taeda, P. echinata*

Zusammenfassung

Neunundzwanzig Einzelbaum-Auslesen von *Pinus taeda, Pinus echinata* und vermuteten Hybriden aus natürlichen Populationen wurden im am weitesten westlich gelegenen Gebiet der Verbreitung der Art (Ost-Texas) durchgeführt. Die Protein-Analyse ergab 13 elektrophoretisch nachweisbare Phänotypen, die mit der Reaktion der frei abgeblühten Familien nach Inokulation mit *Cronartium fusiforme* verglichen wurden. Die Ergebnisse lassen annehmen, daß Introgression der beiden Kiefern-Arten stattgefunden hat und die Morphologie des Mutterbaumes und elektrophoretische Phänotypen brauchbare Kriterien für die Voraussage der Reaktion von Nachkommenschaften auf fusiform-Rost in sympatrischen *P. taeda- P. echinata*-Populationen in Ost-Texas darstellen.

Indroduction

Loblolly pine *(Pinus taeda* L.) and shortleaf pine *(P. echinata* MILL.) are sympatric species capable of producing viable, hybrid progeny (LITTLE and RIGHTER, 1965). The greater resistance of western loblolly pine populations to infection by fusiform rust (*Cronartium fusiforme* HEDGC. and HUNT ex CUMM.) has been attributed to introgression with shortleaf pine, considered almost totally resistant to the rust pathogen (HARE and SWITZER, 1969; POWERS, 1975; WELLS and WAKELY, 1966; ZOBEL, 1953). Studies of wild populations located in East Texas have shown that reproductive isolation may vary temporally when local conditions permit an overlap in pollination periods (DORMAN and BARBER, 1956; HICKS et al. 1972). Attempts to identify a substantial population of the anticipated hybrid phenotypes resulting from natural hybridization have met with limited success (COTTON et al, 1975; WELLS et al, 1977).

Earlier papers have reported results from our study of natural variation, rust resistance, and putative hybridization (BHAT and HICKS, 1976; FLORENCE and HICKS, 1976) among naturally regenerated populations of loblolly and shortleaf pine in East Texas. These results were based on morphometric analyses of maternal and progeny phenotypes, and levels of fusiform rust resistance among the open-pollinated families. The present paper attempts to further substantiate the authors' previous observations that introgression may persist in East Texas populations of loblolly and shortleaf pine, though F_1-hybrid phenotypes may occur at low frequencies.

Materials and Methods

Sampling procedures have been reported elsewhere (BHAT and HICKS, 1976; FLORENCE and HICKS, 1976; HICKS, 1973). Briefly, the original sampling of maternal trees was systematically biased toward selecting three phenotypic groups classified on the basis of hybrid index scores (ANDERSON, 1949; HICKS, 1973) on a scale of 0.0 - 25.0. Shortleaf pine was grouped into the interval from 0.0 - 5.5; putative hybrids 5.6 - 12.5; and, loblolly pines from 12.6 - 25.0. These partitionings were based upon results of multivariate cluster analysis of East Texas collections reported by COTTON et al (1975).

Of the original 45 trees selected from four wild stands within an 80 km radius of Nacogdoches, Texas, USA, sufficient viable seed for protein electrophoresis remained from only 29 maternal trees. In general, the loblolly pines were found on more mesic sites and the shortleaf and putative hybrid pines on more xeric, upland sites. Because cones, needles, and vegetative buds were collected throughout the upper portion of the crown, collections were expedited by sampling in fresh logging slash. This accessibility was later offset by the inability to return for additional seed cones in subsequent years.

Disc polyacrylamide electrophoresis was performed following procedures outlined in Bull. 565, E-C Apparatus Corp., St. Petersburg, Florida USA. Staining and destaining techniques for general protein banding profiles were modified after Chramback et al. (1967). Banding intensities were recorded using a scanning densitometer at 570 nm. Maternal protein phenotypes were determined from the combined complement of several haploid megagametophytes. Protein extractions were modified after HARE and SWITZER (1969).

Fusiform rust inoculations were provided by the U. S. Forest Service rust testing facility at Bent Creek, North Carolina (LAIRD and PHELPS, 1975). Fusiform rust infection (percent seedlings per family producing stem galls) was determined from 60—120 seedlings per open-pollinated family 9-months following inoculation (inoculum source: Livingston Parrish, Louisiana, USA).

Results and Discussion

Table 1 summarizes the results observed among the 29 trees assayed in this study. Six of 22 protein bands(designated A-V) provided sufficient variation among the three morphological groups to warrant their use as diagnostic markers. The six variable proteins were observed in 13 unique phenotypes. Band positions B_e, I_e, and M_e were determined to be more characteristic of shortleaf pine,

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while C_t, J_t, and L_t were more indicative of loblolly pine (subscripts: $_e$ = *echinata* and $_t$ = *taeda*). Also shown in *table 1* are the maternal morphological indices (HI = hybrid index) and rust infection scores (percent) of the open-pollinated progeny for each maternal selection (FLORENCE and HICKS, 1976).

Table 1. — The electrophoretic and morphological (HI) phenotypes for the 29 mother trees and their distribution among the three groups. The percent infection column is derived from the open-pollinated families, nine months after inoculation (L = loblolly pine; I = putative hybrids; S = shortleaf pine). The "X" indicates presence of a protein band.

		Protein Band Phenotypes (Maternal)						(Progeny)
Tree No.	HI[1]	B_e[2]	I_e	M_e	C_t[3]	J_t	L_t	% Rust Infection
L-1	19.7				X	X	X	52
2	19.2				X	X	X	67
3	20.9				X	X	X	58
4	19.2				X	X	X	58
5	20.1				X	X	X	30
6	20.0				X	X	X	48
7	19.2				X	X	X	62
8	17.7	X			X	X	X	63
9	17.6	X			X	X	X	44
Group Means	19.3							53.5
I-1	6.4			X		X	X	1
2	8.0	X		X	X	X	X	2
3	7.8		X	X	X		X	14
4	7.3	X		X	X	X	X	9
5	8.0	X	X	X	X	X	X	21
6	6.6		X	X	X		X	14
7	12.4			X	X	X	X	38
8	8.2		X	X	X		X	14
Group Means	8.1							14.0
S-1	3.2	X	X	X			X	20
2	4.3	X		X				15
3	5.4		X				X	11
4	3.4		X	X	X			10
5	4.4	X	X	X	X			22
6	4.2	X		X				16
7	4.8	X	X	X				8
8	1.5	X	X	X	X			6
9	3.4		X	X	X			7
10	3.2	X	X	X	X			16
11	4.1	X	X	X			X	18
12	5.0		X	X	X		X	10
Group Means	3.9							13.2

[1]/HI = hybrid index. Determined from needle length (n = 17), number of needles/fascicle (n = 31), fascicle sheath length (n = 10) terminal bud width (n = 53) cone length (n = 26); total measurements per individual = 137.
[2]/"e" subscript = *echinata* (shortleaf). See text.
[3]/"t" subscript = *taeda* (loblolly). See text.

Some of the more striking features that were noted among the protein profiles in *table 1* are: (a) band positions I_e and M_e were not observed among those trees morphologically identified as loblolly pine; (b) band position J_t is absent from any of those selected as shortleaf pine; however, (c) all three of these bands were recovered in various combinations among the putative hybrid group. It is difficult to draw any firm conclusions from these observations due to the potential sampling error and our inability to confirm the inheritance of the bands detected. Speculation alone permits the suggestion that if these proteins are inherited in a codominant, additive fashion e. g. as allelic isozymes detected by electrophoresis (GOTTLIEB, 1977); it would be predicted that gene exchange between two parental species should produce progeny exhibiting unique hybrid complements from the parental populations. Putative hybrid number I—5 *(Table 1)* illustrates this most easily by its protein phenotype exhibiting all six of the diagnostic bands. It is not, however, inferred that tree I—5 represents the protein phenotype indicative of a loblolly × shortleaf F_1-hybrid pine.

Conclusions regarding specific relationships between any of the protein banding patterns and correlated levels of fusiform rust resistance must be approached with caution. Tentatively, it could be suggested that protein banding profiles containing the I_e and M_e bands indicative of shortleaf pine in combination with any of the three prevalent loblolly pine proteins (C_t, J_t, L_t) are candidates as markers of past hybridization and potentially intermediate levels of rust susceptibility. Conclusive evidence must come from more intensive sampling of the parental populations and controlled genetic experiments. Preferably, these experiments would also include protein or isozyme analyses of the rust pathogen itself.

It has been observed in studies utilizing artificially pollinated loblolly and shortleaf pine and their hybrid recombinants that resistance to fusiform rust is under genetic control and that pollination periods tend toward the shortleaf parent (LAFARGE and KRAUS, 1975; POWERS and DUNCAN, 1976). There are also strong indications that increased rust resistance among shortleaf and loblolly hybrids may be combined with growth rates equalling or exceeding either parental species (LAFARGE and KRAUS, 1977). Results from this study and those reported by BHAT and HICKS (1976) suggest that similar processes may be associated with these putative hybrid populations in East Texas. The tentative basis for concluding that introgression is implicated and early backcrosses may favor the shortleaf parent in the East Texas region is formulated by observing that: (a) intermediate, multivariate morphologies have been identified; (b) average rust resistance among the intermediate, putative hybrids is comparable to the more resistant shortleaf parent; (c) two of three protein phenotypes strongly associated with loblolly pine morphology appear to have entered the shortleaf pine population via the putative hybrid group; and, (d) seedling height growth among the putative hybrid group of the present study has been found to exhibit moderate levels of heterosis, exceeding either parental group (BHAT and HICKS, 1976).

These results of protein variation and resistance to fusiform rust among an array of pine phenotypes in East Texas offer a point of departure for more detailed genetic study of host-rust relationships among wild populations of loblolly and shortleaf pines. When consideration is given of the time and resources required to record 137 morphological measurements (HICKS, 1973) needed to classify each individual's morphological phenotype with confidence, biochemical markers offer a more expeditious opportunity for studying bio-systematic relationships. These results and geographic trends among loblolly pine allozymes sampled recently from populations east and west of the Mississippi River (FLORENCE and RINK, 1979) encourage further investigations into the coevolution of pine: pest relationships.

Conclusions

Electrophoretic analysis of total proteins from the maternal gametophytic tissues yielded sufficient heterogeneity between the two pines enabling reasonable characterization of prevalent species banding profiles for loblolly and shortleaf pines. Morphologically intermediate (putative hybrids) pines exhibited unique protein phenotypes com-

posed of diagnostic markers attributed to the two parental species.

Infection by fusiform rust inoculum of the open-pollinated progeny among the 29 maternal tree selections showed the loblolly pine to be considerately more susceptible than the shortleaf and putative hybrid pines. These observations together with results from other studies indicate that genetic introgression has likely been an important component during the evolution of these two species, but possibly more so in the western perifery of their sympatry in East Texas.

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